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protease and an integration cassette comprising a gene encoding a mutant high alkaline protease; and

b) growing said mutant alkalophilic Bacillus host under conditions whereby said mutant high alkaline protease is expressed.

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45. (Once Amended) The method of Claim 41 wherein said gene encoding the wild-type alkaline protease in said mutant alkalophilic Bacillus host has been deleted by homologous or illegitimate recombination.

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- 47. (Once amended) The method of Claim 41 wherein said integration cassette is integrated into the genome of said mutant alkalophilic Bacillus host.
- 48. (Once amended) A method of obtaining a non-reverting mutant alkalophilic Bacillus strain having a reduced level of a wild-type high alkaline protease, said method comprising the steps of:
- a) transforming an alkalophilic Bacillus strain comprising a gene encoding the wild-type alkaline protease with a cloning vector comprising DNA encoding a replication function and 5' and 3' flanking non-coding regions of said gene encoding the wild-type high alkaline protease but not the coding region of said gene encoding the wild-type high alkaline protease gene, wherein a sufficient amount of said 5' and 3' flanking non-coding regions is present to provide for homologous recombination with the indigenous gene encoding the wild-type alkaline protease of said alkalophilic Bacillus strain whereby transformants having a reduced level of said wild-type alkaline protease are obtained;
- b) growing said transformants under conditions whereby the replication function encoded by said cloning vector is inactivated; and
- c) isolating transformants having a reduced level of the wild-type alkaline protease.



50. (Once amended) A mutant alkalophilic Bacillus strain producing a mutant high alkaline protease and no detectable level of a wild-type high alkaline protease, wherein said mutant alkalophilic Bacillus strain is obtained by growing an alkalophilic Bacillus

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strain which is incapable of producing said wild-type high alkaline protease transformed with a plasmid expression vector comprising said mutant high alkaline protease gene.

Please add the following new claims:

- 54. A method for the production of a mutant high alkaline protease, said method comprising the steps of:
- a) obtaining an alkalophilic Bacillus host selected from the group consisting of Bacillus novo species PB92 and its derivatives wherein said derivatives retain the characteristics of Bacillus novo species PB92 and said alkalophilic Bacillus host is incapable of producing a wild-type high alkaline protease, and comprises a chromosomal deletion of the gene encoding an the wild-type high alkaline protease;
- b) transforming said alkalophilic Bacillus host with an integration cassette comprising a gene encoding a mutant high alkaline protease, wherein said gene encoding the mutant high alkaline protease comprises a replacement of at least one amino acid residue in the nucleotide sequence encoding the wild type protease of Bacillus novo species PB92 or derivative thereof to obtain a non-reverting mutant alkalophilic strain; and
- c) growing said mutant alkalophilic Bacillus host under conditions whereby said mutant high alkaline protease is expressed.
- 55. The method according to claim 54 wherein the replacement is at an amino acid residue position selected from the group consisting of positions of 160, 216 and 212.

